Fungal and bacterial contaminants associated with spoilage of spawn

ABSTRACT

Contamination has been a major problem associated with spawn of mushroom. In this study, the contaminating microorganisms in the spawn were isolated, identified and described. Results indicated that majority of microorganisms causing spawn contamination were mainly fungal pathogens; a very low percentage of bacterial contamination was observed. The fungal species causing contamination were *Fusarium chlamydosporum, Phoma exigua, Aspergillus niger, Penicillium chrysogenum,Aspergillus fumigatus* and *Fusarium pallidoroseum.* Only one bacterial contaminant i.e. *Bacillus* sp. was reported under study. *Aspergillus fumigatus* was the major fungal contaminant and *Bacillus* sp. was the only bacterial contaminant isolated from spawn incubation room environment, whereas from spawn inoculation room contaminants isolated were*Fusarium pallidoroseum* and *Staphylococcus* sp.

Key words: Fungal contaminants, bacterial contaminants,spoilage,spawn characterization

Introduction

Spawn plays an important role in the mushroom industry because the failure or success of mushroom cultivation depends upon the availability of pure culture spawn.Thesuccessofmushroomcultivationanditsyield depend to a large extent on the vigour and quality of spawn used (Bahl 1984). The yield and quality of the spawn is governed mainly by the genetic makeup of the strainandthetechnologyincludingthesubstratesusedin spawnproduction.Spawnqualityiscountedasthemost important aspect of mushroom Quality is also one ofthe most important factor in the production of ediblemushroom. In India, 15-20 percent losses due to spawn spoilage have been reported. Hence the spoilage of spawn by some fungal and bacterial contaminants is consideredtobeoneofthebiggestconstraintsfacedby spawn productionlaboratories.

Comment [A1]: Must be documented!

MATERIALS AND METHODS

In order to know the type and extent of contamination, various microorganisms mainly fungi, and bacteria were isolated from the spawn bags /bottles and wheat grains which were used as substrate for spawn production in different months. Spawn bags were examined during their incubation in spawn incubation room. Number of bags inoculated during each month and the numbers of spawn bottles showing fungal and bacterial contaminations were separately recorded.

Based on the data recorded every month, the percentage of spawn bags contaminated in different months of the year were determined.

Isolation of microorganism from the laboratory environment (Spawn incubation room, Spawn inoculation room)

The Petri plates containing potato dextrose agar medium/ nutrient agar medium was exposed for one hour, quarterly for a period of one year at different locations in the laboratory. After the exposure, the Petri plates were incubated at 25$\pm$ 1^0C for seven to ten days. The colonies of various microorganisms which were seen in the Petri plates, were transferred onto potato dextrose agar medium or nutrient agar medium in case of fungi and bacteria, respectively for getting the pure culture.

Comment [A2]: Where did you inspire? In literature? Please indicate the author!

RESULTS AND DISCUSSION

The spawn bags were examined for a period of two-weeks to detect the growth of contaminants. During each month, per cent contamination of spawn bags caused by fungal and bacterial contaminants were recorded at spawn production and research laboratory, Chambaghat. The contaminants isolated from the spawn bags in different months and extent of spoilage have been presented in the Table 1.The results reveal that maximum contamination was observed in the month of August (18.33%) followed by September (17.67%) and July (16.67%) while minimum contamination was observed in the month of January (8.67%). It was also observed that majority of microorganisms causing spawn contamination were mainly fungal pathogens; a very low percentage of bacterial contamination was observed. The fungal species causing contamination were *Fusarium chlamydosporum, Phoma exigua, Aspergillus niger, Penicillium chrysogenum,Aspergillus fumigatus* and *Fusarium pallidoroseum.* Only one bacterial contaminant i.e. *Bacillus* sp. was reported under study.

Suman and Jandaik (1992) also reported that the frequency of occurrence of various fungal and bacterial microflora was high during the summer months (March to August) whereas, their incidence started decreasing with the start of winter months (September to February).Hence, it may be concluded that the weather conditions prevailing during the summer and rainy months were very congenial for the rapid multiplication and growth of the contaminant microflora resulting in maximum spoilage of spawn. Mazumder *et al.* (2005) also studied seasonal variation in microbial contamination of *Pleurotus ostraetus* spawn caused by various fungal and bacterial contaminants and observed that contamination was highest (28.57%) during monsoon season followed by pre-monsoon(21.90%) which is similar to our findings. Moorthy and Mahanan (1996) observed that the highest spawn contamination of *P. sajor- caju* was found during June to September. An increasing trend in total per cent contamination (22.85 to 31.42%) was observed starting from May to August, 2000 after which it declined slowly (22.85 to 8.57%) from September, 2000 to January, 2001. The highest spawn contamination of *P. sajor- caju* was found during June to September. It can be inferred from above discussion that wet and humid weather conditions particularly during rainy season were responsible for causing heavy losses in spawn. It was also observed that *Aspergillus* was the major contaminant.

Table 1 Occurrence of different microorganisms in different months of the year 2015

Month	Total no. of Bags	No. of Contaminated Bags	Contamination (%)	Contaminants Isolated
January	300	26	8.67	*Aspergillusniger, Bacillus* sp.
February	300	27	9.00	*Aspergillusniger, Aspergillus fumigatus,Penicilliumchrysogenum ,Bacilllus* sp.
March	300	30	10.00	*Aspergillusniger, Aspergillus fumigatus, Bacillus* sp.
April	300	32	10.67	*Penicilliumchrysogenum, Aspergillusniger*

				Bacillus sp.
May	300	34	11.33	*Aspergillusniger, Aspergillus fumigatus, Penicilliumchrysogenum, Bacillus* sp.
June	300	45	15.00	*Aspergillusniger, Aspergillus fumigatus, Penicilliumchrysogenum, Bacillus* sp
July	300	50	16.67	*Fusarium pallidoroseum,Phoma exigua ,Aspergillusniger, Aspergillus fumigatus Penicilliumchrysogenum , Bacillus* sp.
August	300	55	18.33	*Staphylococcus* spp., *Aspergillus fumigatus, Penicilliumchrysogenum,,Bacillus* sp *,Fusarium pallidoroseum,Phoma exigua, Aspergillusniger*
September	300	53	17.67	*Aspergillus fumigatus,Penicilliumchrysogenum ., Aspergillusniger ,Bacillus* sp.
October	300	35	11.67	*Penicillium chrysogenum, Aspergillusniger,Bacillus* sp.
November	300	31	10.33	*Aspergillusniger , Bacillus* sp.
December	300	29	9.35	*Aspergillus fumigatus , Aspergillusniger,*

				Bacillus sp.

MICROORGANISMS IN THE LABORATORY ENVIRONMENT (SPAWN INCUBATION ROOM, SPAWN INOCULATION ROOM)

Different types of contaminants isolated from laboratory environment are given in Table 2 and 3. It is clear that *Aspergillus fumigatus* was the major fungal contaminant and *Bacillus* sp. was one bacterial contaminant isolated from spawn incubation room environment , whereas from spawn inoculation room contaminants isolated were *Fusarium pallidoroseum* and *Staphylococcus* sp. In general, fungal contaminants were predominant irrespective of the period of isolation as well as locations. Maximum number of fungal and minimum number of bacterial colonies were recorded on agar plates. Bacterial contaminants were identified on the basis of morphological and cultural characteristics as well as biochemical tests which are being presented in Table 3. Yeast and actinomycetes were not observed during the period under investigation. According to Oxaley (1985) and Earanna (1991) the spawn contamination was assumed to be caused by the air-borne microflora present inside the inoculation room. During the transfer of the mother spawn into the autoclaved bags, air carrying air-borne microflora might enter into the bags quickly and lead to contamination of spawn during incubation. Similar kind of results have been recorded in the present study.

Table 2 Isolation of fungal contaminants from spawn laboratory environment

SN	Isolation of fungal contaminant from Spawn Laboratoy	Diametric growth after 6 days (cm)	Colony Colour	Conidiophores	Species Identified
1.	Spawn incubation	5-7cm	Blue grey	Conidiophore	***Aspergillus***

	room			are smooth, some shade of brown	***fumigatus***
2.	Spawn inoculation room	6-7cm	Grayish white to cream	Macrocondia on phialides are present	***Fusarium pallidoroseum***

Table 3 Isolation of bacterial contaminants from spawn laboratory environment

Isolation of bacterial contaminant from Spawn Laboratory	**Morphological Characteristics**		**Cultural Characteristics**	**Biochemical Tests**	**Bacterial Genera Identified**
Spawn incubation room	**Shape**	**Gram staining**			
	Rod	+ve	Colonies are dry, flat and irregular with lobate margin	Catalase test (+ ve)	***Bacillus* sp.**
Spawn inoculation room	Cocci	+ve	Clonies are slimy and yellow in colour	Catalase test (-ve)	***Staphylococcus* sp.**

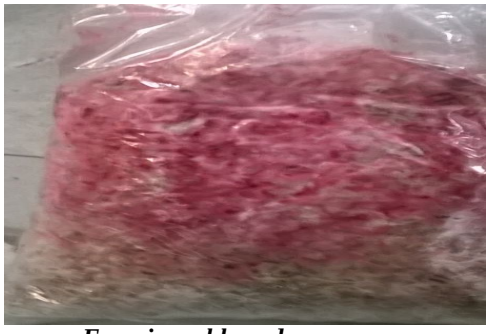
Suman and Jandaik (1992) while studying the microbial contaminants of spawn of *Agaricus bisporus* reported that the prime sources of contaminantion were unsterilized wheat grains and microbes present in the environment of spawn laboratory. They isolated and identified twenty four species of mould and one species of bacteria and yeast in commercial spawn of *Agaricus bisporus.* They also isolated *Aspergillus niger*, *Penicillium*, *Mucor* and *Bacillus* sp. from different locations in the laboratory which are similar to present findings. The present observations pertaining to isolation of various contaminants from spawn bags are in agreement with observations of Stoller(1962) and Bitner(1972) who reported similar findings

Comment [A3]: How did obtain this results?

and isolated various microorganisms. Other workers (Thapa *et al*.1976 and Bahl 1989) have also reported various fungal ,bacterial and yeast contaminants of spawn.

Characterization and identification of isolated bacteria *Staphylococcus* sp. They are facultatively anaerobic, Gram positive, coccus, which appear as grape-like clusters when viewed through a microscope, and has round, usually golden-yellow colonies. It lacks endospore formation and are non-motile.

Isolate no. 1

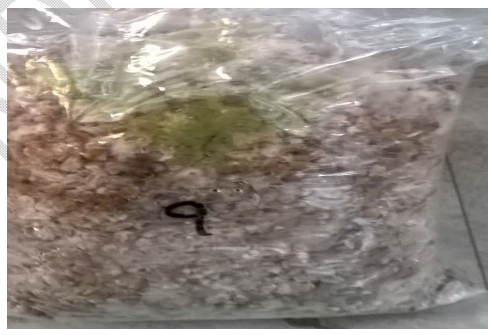


Fusarium chlamydosporum

Isolate no. 2

Aspergillus niger

Isolate no. 3



Penicillium chrysogenum

Plate 1: Growth of contaminants in spawn

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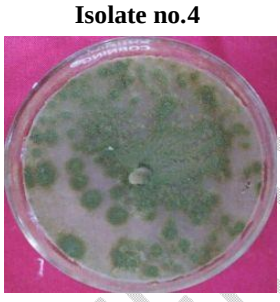
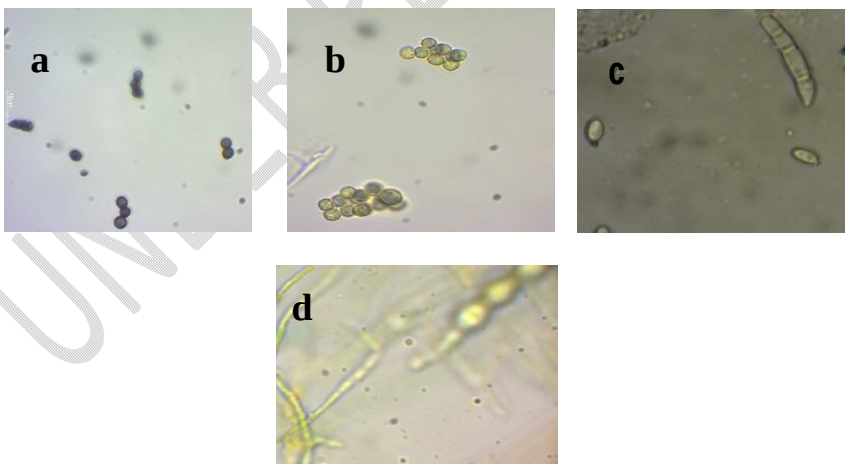
Fusarium chlamydosporum *Phoma exigua* *Aspergillus niger*



Penicillium chrysogenum

a. Cultures of different fungal contaminants



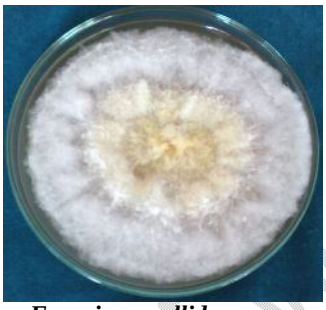
b. Conidia of (a) *Aspergillus niger* , (b) Penicillium *chrysogenum* ,(c)*Fusarium chlamydosporum* , (d) *Phoma exigua* (hyphae)

Plate 2. Culture and morphology of Fungus isolated from spawn bags

Isolate no. 5

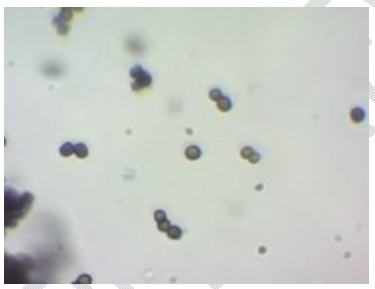
Aspergillus fumigatus

Isolate no.6



Fusarium pallidoroseum

a. Cultures of *Fusarium pallidoroseum* and *Aspergillus fumigatus*



b. Conidia of *Aspergillus fumigatus*

***Bacillus* Sp.**

***Staphylococcus*Sp.**

c. Cultures of *Bacillus* and *Staphylococcus* species

Plate 3. Cultures of Fungal and Bacterial Contaminants isolated from spawn laboratory environment

CONCLUSIONS

Isolation of various microorganisms from contaminated spawn bottles / bags revealed that fungi were the largest group of microorganisms isolated over a period of one year followed by bacteria.*Fusarium chlamydosporum, Phoma exigua, Aspergillus niger, Aspergillus fumigatus, Penicillium chrysogenum* were the major fungal contaminants isolated from spawn bags and were identified by National Centre of Fungal Taxonomy, New Delhiand among bacterial contaminants, only one species of *Bacillus* was isolated. *Phoma exigua* was reported for the first time. One year data collected on monthwise per cent contamination revealed that maximum contamination was observed during the month of August(18.33%) followed by the month of September (17.67%) and minimum was observed in the month of January (8.67%). Spawn laboratory environment was found to be heavily contaminated by the microorganisms. Among fungal contaminants *Aspergillus fumigatus, Fusarium pallidoroseum* were isolated and among bacterial contaminants *Staphylococcus* sp.and *Bacillus* sp. were isolated from spawn laboratory environment and were identified.

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